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Exobasidium ferrugineae sp. nov., associated with hypertrophied flowers of *Lyonia ferruginea* in the southeastern USA

Aaron H. Kennedy[1], Nisse A. Goldberg[2] & Andrew M. Minnis[3*]

[1]*National Identification Services, USDA-APHIS-PPQ-PHP, 10300 Baltimore Ave., B 580, Beltsville, MD, 20705, USA*
[2]*Jacksonville University, Dept. of Biology and Marine Science, 2800 University Blvd. North, Jacksonville, FL 32211, USA*
[3]*Center for Forest Mycology Research, Northern Research Station, USDA-Forest Service, One Gifford Pinochet Drive, Madison, WI 53726, USA*
* Correspondence to: *amminnis@fs.fed.us*

Abstract — *Exobasidium ferrugineae,* associated with hypertrophied flowers and less commonly leaves of *Lyonia ferruginea* (rusty staggerbush), is formally described here as a new species. Morphological and DNA sequence (ITS, nLSU) data are provided. Phylogenetic analyses confirm that it is not conspecific with any species of *Exobasidium* represented by existing DNA sequence data. A key to North American species of *Exobasidium* on *Lyonia* is presented.

Key words — *Basidiomycota*, *Ericaceae*, *Exobasidiales*, *Exobasidiomycetes*, plant pathogen

Introduction

Exobasidium Woronin (*Exobasidiales*, *Exobasidiomycetes*) is a basidiomycetous genus associated with diseases of ericaceous plants commonly characterized by formation of galls on leaves, shoots, and flowers (Burt 1915, Savile 1959, Nannfeldt 1981). Early authors named species on the basis of symptomatology and host association, whereas monographers, including Burt (1915) and Savile (1959), advocated broader taxonomic concepts. These authors suggested that symptoms were variable, overlapping, and dependent on time and environmental conditions. Furthermore, fungal morphology was not definitive for species recognition and usually poorly known, and host associations are not supported by inoculation and cross-inoculation experiments. According to these authorities, only a small number of species were accepted in *Exobasidium* and the citation of numerous synonyms was deemed unreliable (Burt 1915, Savile 1959). Nannfeldt (1981) reviewed a large amount of data and literature

with special emphasis on life cycles and symptoms. He concluded that species of *Exobasidium*, though often quite similar morphologically, are rigidly host-limited taxa. Recent molecular studies including those by Begerow et al. (2002) and Piątek et al. (2012) support Nannfeldt's hypotheses on the importance of host association and host specificity.

In April 2011, an unusual flower of *Lyonia ferruginea* (Walter) Nutt. (rusty staggerbush; *Ericaceae*) that was infected and highly transformed by a fungus was found in Florida. Microscopic examination confirmed that a species of *Exobasidium* was growing on the hypertrophied flower. This species was subsequently characterized with morphological and DNA sequence (ITS, nLSU) data. Careful comparison with known species using historical literature, herbarium specimens, and phylogenetic analyses revealed that it represents an undescribed taxon that is described herein. A key to North American species of *Exobasidium* on *Lyonia* is presented in order to facilitate identification and promote further study of *Exobasidium* from North America.

Materials & methods

Morphology

Observations and measurements of microscopic characters were made from crush mounts of material scraped from the hymenial surface that covered the flower. Material was rehydrated and viewed in 3% KOH, and occasionally phloxine solution was used to improve the visibility of fungal structures (Largent et al. 1977). Sterigmata are not included in length measurements of basidia. Voucher specimens were deposited into the U.S. National Fungus Collections (BPI).

Molecular data collection & phylogenetic analysis

DNA was extracted from scraped hymenial surface material from the infected flower of *L. ferruginea* (N.A. Goldberg, s.n.; BPI 882571) using Qiagen's DNeasy Plant Mini Kit (Germantown, MD). Ribosomal DNA sequence data were generated from the internal transcribed spacer region (ITS; ITS1, 5.8S, ITS2) and the nuclear encoded large subunit (nLSU) using the primer pairs ITS5/ITS4 (White et al. 1990) and NL1/NL4 (O'Donnell 1992), respectively. GoTaq (Promega, Madison, WI) and associated standard reagents were used for PCR following the manufacturer recommendations including 2.0 mM MgCl. Standard thermal cycling was used including an annealing temperature of 55°C for the ITS and 50°C for the nLSU. PCR products were fluorescently labeled using respective forward and reverse PCR primers and the BigDye v3.1 dye terminator kit (Applied Biosystems; Foster City, CA). The purified products were then sequenced on an ABI 3730 automated DNA sequencer. Electropherograms were edited in Geneious Pro v.5 (Drummond et al. 2010). Resulting consensus sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov).

BLAST searches were conducted independently with the ITS and nLSU sequences against GenBank using the megablast algorithm. Taxon sampling for phylogenetic analyses was primarily determined by selecting the top hits from each search, sorted by Max Score; the phylogenetic results of Piątek et al. (2012); and morphological and

host association similarity. The ITS and nLSU sequences for each taxonomic unit were derived from the same isolate.

Multiple sequence alignments of the ITS and nLSU were conducted using MUSCLE v3.6 (Edgar 2004) within Geneious Pro v5 (Drummond et al. 2010) and then concatenated. Although nLSU data were available for all taxa included in the concatenated alignment, ITS data were unavailable for *Exobasidium karstenii*, *E. oxycocci*, *E. pieridis*, and *E. sundstroemii* and therefore treated as missing data. The best-fit model of DNA sequence evolution was independently determined for ITS and nLSU in MrModeltest v2.2 (Nylander 2004) according to the Akaike Information Criterion (AIC; Posada & Buckley 2004). The GTR+I+G model was selected for both data sets and implemented in the following analysis. The concatenated alignment was analyzed phylogenetically with Bayesian Inference (BI) in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) leaving all other parameters as default. The posterior probability (pp) distribution of trees was estimated from two independent Markov Chain Monte Carlo (MCMC) simulations of 3 million generations, sampling trees every 100 generations until the standard deviation of split frequencies reached 0.01. The burn-in was determined using the program Tracer v1.5 (Rambaut & Drummond 2007). A 50% majority rule consensus tree was constructed from the remaining trees in FigTree v1.3.1 (Rambaut 2009).

The concatenated alignment was also analyzed using Maximum Likelihood (ML). This analysis was conducted using RAxML (Stamatakis 2006) via the on-line CIPRES (Cyberinfrastructure for Phylogenetic Research) Science Gateway V. 3.1 (http://www.phylo.org/sub_sections/portal/; Miller et al. 2010) with the RAxML-HPC BlackBox (7.2.8) tool. One thousand bootstrap (bs) replicates were run and all other parameters were left as default.

Results

Alignment of the ITS resulted in a matrix of 595 nucleotide positions, 296 of which were variable (49.7%) and 156 of which were parsimony informative (26.2%). Alignment of the nLSU resulted in a matrix of 555 nucleotide positions, 146 of which were variable (26.3%) and 71 of which were parsimony informative (12.8%).

The phylogenies resulting from the BI and ML analyses were entirely congruent with the exception of the *E. pieridis*/*E. ferrugineae* clade, which occupied a different position in the most optimal ML tree vs. the BI consensus tree (Fig. 1). This is an insignificant observation considering the position of this clade is entirely unsupported in the ML bootstrap tree and received very low posterior probability in the BI tree. Each analysis revealed high support (pp = 0.99, bs = 99%) for the *E. pieridis*/*E. ferrugineae* clade, revealing a close relationship between these species and demonstrating that *E. ferrugineae* is a species distinct from *E. vaccinii* (Fig. 1).

The relationship between *Exobasidium ferrugineae* and *E. karstenii* was of particular interest because *E. karstenii* was among the top hits for nLSU.

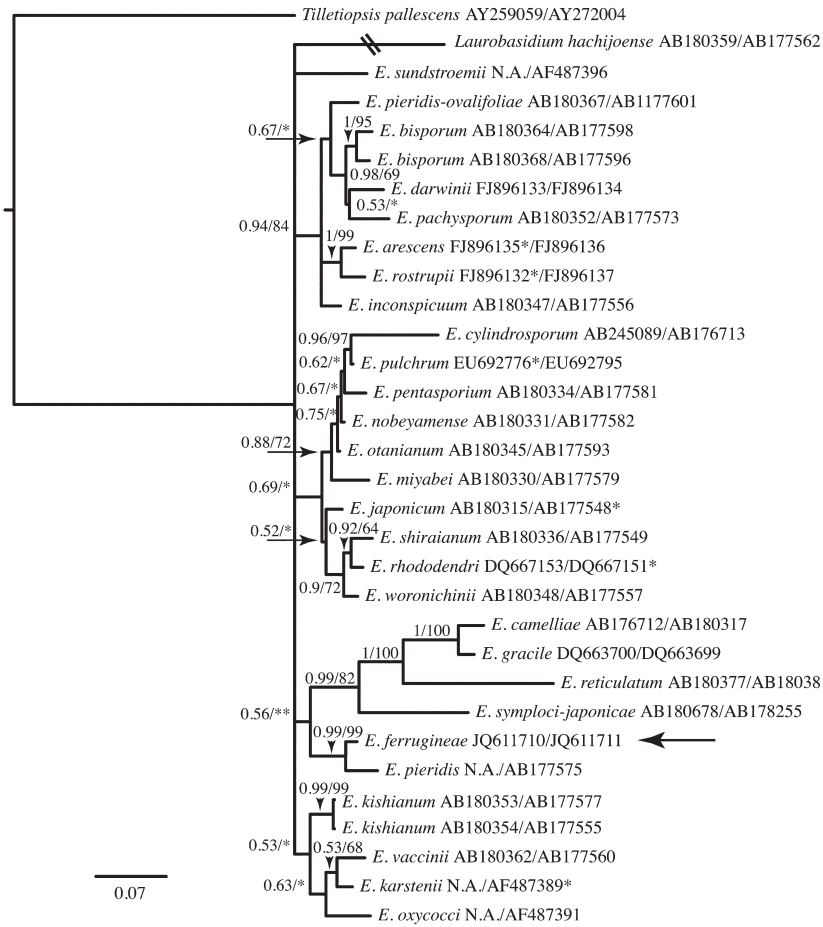

Fig. 1. A phylogenetic summary of relationships among *Exobasidium* species resulting from Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of a combined ITS/nLSU data matrix. This phylogram is the resulting 50% majority rule consensus tree from the BI analysis with corresponding posterior probabilities (pp) and appended bootstrap (bs) values from the ML analysis on each branch (pp/bs). One asterisk indicates a bs value < 50%, while two asterisks indicates a clade that was not recovered in the most optimal ML tree. The hatch marks on the "*Laurobasidium hachijoense*" nom. prov. in GenBank [≡ *E. hachijoense*] branch indicate that it was shortened for presentation purposes from 8.5 times the scale bar. GenBank accession numbers are provided after each taxon name (ITS/nLSU). Asterisks here indicate top BLAST hits for the associated marker. The arrow indicates the position of *E. ferrugineae* (holotype, BPI 882571).

However, our phylogenetic results suggested, albeit with very weak support (pp=0.53, bs = 68), that *E. karstenii* is most closely related to *E. vaccinii*. Again, the relationship of this clade to others was unsupported.

Taxonomy

Exobasidium ferrugineae Minnis, A.H. Kenn. & N.A. Goldberg, **sp. nov.** Figs. 2–4

MycoBank 564773

Characterized by its association with hypertrophied flowers of *Lyonia ferruginea*.

type: USA. Florida: Jacksonville, Jacksonville Arboretum, Rosemary Ridge Trail, on flower of *Lyonia ferruginea*, 18.IV.2011, leg. N.A. Goldberg, s.n., (**Holotype** BPI 882571; GenBank accession nos., ITS JQ611710, nLSU JQ611711).

Etymology: The species epithet is derived from the host, *Lyonia ferruginea*.

Hymenium consisting of a thin white layer on hypertrophied flowers having all parts proportionally enlarged, infected flowers up to approx. 6 cm long, or less commonly on leaves that may also be hypertrophied. Basidia 37–54.5 × 6.5–8.5 µm, cylindrical to cylindrical-clavate, 3–5 sterigmate, thin walled, hyaline, often with granular contents. Sterigmata approx. 4.5–6.5 × 1–2 µm, typically slightly wider towards base. Basidiospores 13.5–19 × 4–6.5 µm, ellipsoid to musiform, with a prominent apiculus, walls thin and smooth, initially with one more or less median septum at maturity, later with up to six septa, hyaline. Germination via germ tubes at both ends of basidiospores. Conidia 4–9.5 × 1–2.5 µm, bacilliform, ellipsoid or slightly clavate, walls thin and smooth, aseptate, hyaline, contents often with a few scattered guttules.

Habitat & distribution— This species is known from hypertrophied flowers and less commonly hypertrophied leaves of *Lyonia ferruginea* (*Ericaceae*). It is known from Florida and Georgia, and is predicted to occur in South Carolina, and throughout the narrow, native range of its host (USDA 2011).

Additional specimens examined— **USA. Florida:** on flower of *Lyonia ferruginea*, leg. Chapman, s.n., Mo. Bot. Gard. Herb. 44409 (BPI 292003 as *Exobasidium vaccinii*); Dunedin, on flower of *Lyonia ferruginea*, 16.IV.1900, leg. S.M. Tracy, SMT 7159, Mo. Bot. Gard. Herb. 4962 (BPI 292004 as *E. vaccinii*); Jacksonville, Jacksonville Arboretum, Rosemary Ridge Trail, on leaves of *Lyonia ferruginea*, 06.V.2011, leg. N.A. Goldberg, s.n. (BPI 882572); additional collections on *Lyonia ferruginea* made by N.A. Goldberg from the area of the type locality, Rosemary Ridge Trail: 18.IV.2011 (BPI 882566, BPI 882567, BPI 882568, BPI 882569, BPI 882570, BPI 882580, BPI 882582, BPI 882583), 06.V.2011 (BPI 882573, BPI 882574, BPI 882575, BPI 882576, BPI 882577, BPI 882578, BPI 882579, BPI 882581). **Georgia:** Brunswick, on flower of *Lyonia ferruginea*, 01.VI.1901, leg. W. Trelease, s.n., Mo. Bot. Gard. Herb. 4955 (BPI 292005 as *E. vaccinii*).

Comments— Burt (1915) studied collections of hypertrophied flowers of *Lyonia ferruginea* caused by *Exobasidium ferrugineae* and concluded that the species was conspecific with *E. vaccinii*. Burt (1915) also commented on the fact that the hypertrophied flowers were much larger than those of *L. mariana* found in association with *E. peckii* Halst., which he also concluded was a synonym of *E. vaccinii*. However, the results presented here show that *E. ferrugineae* is distinct from *E. vaccinii*. *Exobasidium vaccinii* has been shown to be restricted

Fig. 2. *Exobasidium ferrugineae* associated with hypertrophied flowers of *Lyonia ferruginea*. Note uninfected flowers in upper image for comparison. All flower parts are proportionally enlarged.

to *Vaccinium*, likely just *Vaccinium vitis-idaea* (Nannfeldt 1981, Begerow et al. 2002, Piątek et al. 2012).

Fig. 3. *Exobasidium ferrugineae* associated with hypertrophied leaves of *Lyonia ferruginea*.

Several species of *Exobasidium* are known from *Lyonia* (Farr & Rossman 2011) and eight of these were described originally from this host genus. Five Asian species, *E. kunmingense* Zhen Ying Li & L. Guo (Li & Guo 2009), *E. lyoniae* Zhen Ying Li & L. Guo (Li & Guo 2006), *E. ovalifoliae* Zhen Ying Li & L. Guo (Li & Guo 2008), *E. pieridis* Henn. (Hennings 1903), and *E. pieridis-ovalifoliae* Sawada (Sawada 1931), are distinguished from *E. ferrugineae* by their occurrence on *L. ovalifolia* and their association that is confined to leaf diseases. There are three previously described species on *Lyonia* from North America. *Exobasidium andromedae* Peck (Peck 1874) and *E. fawcettii* Massee (Massee 1908) occur on different hosts than *E. ferrugineae* and are associated with leaf diseases. At times, *E. andromedae* may produce unique bag galls where developing leaves are transformed into hollow bags (Peck 1874, Burt 1915). *Exobasidium peckii* is similar to *E. ferrugineae* as it infects both flowers and leaves (Halstead 1893, Stewart 1896), but it is distinguished by its occurrence on *Lyonia mariana* and a lesser amount of hypertrophy on flowers. Based on our morphological examination of a collection of *E. peckii* (USA: New York, Hicksville, Long Island, on flower of *Lyonia mariana*, 25.VI.1919, leg. G.P. Clinton, s.n. (BPI 292023 as *E. vaccinii*)), this species is nearly indistinguishable morphologically from *E. ferrugineae*. However, *E. peckii* differs in the basidiospores with none having been observed with more than 5 septa along with typically fewer septa per basidiospore and the conidia having a tendency towards an allantoid shape.

Our phylogenetic analyses indicate that *E. ferrugineae* is distinct from all species with existing ITS and nLSU sequence data in GenBank (data were

Fig. 4. *Exobasidium ferrugineae* (holotype, BPI 882571).
A–B. Basidia. C. Basidiospore. D. Conidia. Scale bars are 20 µm for A–B, 10 µm for C–D.

unavailable for species on *Lyonia* including *E. andromedae*, *E. fawcettii*, *E. kunmingense*, *E. lyoniae*, *E. ovalifoliae*, *E. peckii*) including the isolates that are labeled *E. pieridis* and *E. pieridis-ovalifoliae* from *Lyonia neziki*. The close relationship between *E. ferrugineae* and *E. pieridis* is noteworthy because of their highly discontinuous geographic ranges. We suggest that this geographic isolation has led to reproductive isolation and that the relationship between *E. ferrugineae* and *E. pieridis* is a product of the tendency for host specificity in *Exobasidium*, in this case with *Lyonia*, and divergence in host range relative to their recent common ancestor. Therefore, their well-supported sister-relationship found here suggests recent common ancestry, but not conspecificity. Unfortunately, ITS data were unavailable for *E. pieridis*, resulting in missing data for combined analysis. This is significant because the ITS region is more informative than nLSU primarily because it more rapidly accumulates substitutions among species. This is evidenced by the fact that the

ITS region contains twice the number of variable and informative characters among *Exobasidium* species. Therefore, support for this relationship may be artificially high in this combined analysis due to missing ITS data for *E. pieridis*. Further sampling will be needed to address the complex patterns of speciation including potential co-speciation in *Exobasidium* as discussed by Begerow et al. (2002) and Piątek et al. (2012).

Key to *Exobasidium* on *Lyonia* of North America

1. On *L. ligustrina*, with bag galls ..... *E. andromedae*
1. On other species of *Lyonia*, bag galls absent 2
2. On *L. jamaicensis*, with hypertrophied leaves ..... *E. fawcettii*
2. On other species of *Lyonia*, associated with flowers & occasionally leaves 3
3. On *L. mariana*, with hypertrophied flowers relatively small (up to approx. 2 × longer), some conidia allantoid ..... *E. peckii*
3. On *L. ferruginea*, with hypertrophied flowers relatively large (up to approx. 5 × longer), conidia not allantoid ..... *E. ferrugineae*

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